# 9th Croatian-Slovenian-Serbian Symposium on Zeolites

# PROCEEDINGS

Editors

Ivona Nuić

Matjaž Mazaj

Aleksandra Daković

Zagreb, 2021

***Statue of Grgur Ninski (Bishop Gregory of Nin), Split, sculpted by Ivan Meštrović.*** Gregory of Nin, 10th-century Croatian bishop who defended the usage of old Croatian language in liturgical services instead of Latin. According to tradition, after rubbing his big toe, your wish will come true…

Front-page: Panoramic view of historic old town of Split (the palace of Diocletian), waterfront, West coast and Marjan hill

# Proceedings of the 9th Croatian-Slovenian-Serbian Symposium on Zeolites
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# PROCEEDINGS OF THE 9th CROATIAN-SLOVENIAN-SERBIAN SYMPOSIUM ON ZEOLITES

September 23-25, 2021
Split, Croatia

Organizers
CROATIAN ZEOLITE ASSOCIATION
SLOVENIAN ZEOLITE ASSOCIATION
SERBIAN ZEOLITE ASSOCIATION

Under the auspices of
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# 9th Croatian-Slovenian-Serbian Symposium on Zeolites

23rd-25th September 2021, Split, Croatia

| Time | **Thursday, 23. 09. 2021** | Time | **Friday, 24. 09. 2021** | Time | **Saturday, 25. 09. 2021** |
|---|---|---|---|---|---|
| 8:30-9:00 | Registration | | | | |
| 9:00-9:30 | Opening | | | | |
| | *chair Bronić* | | *chair Rakić* | | *chair Vukojević Medvidović* |
| 9:30-10:15 | PL Mintova | 9:00-9:45 | PL Giordano | 9:00-9:45 | PL Migliori |
| 10:15-10:45 | IL Byrne | 9:45-10:15 | IL Žerjav | 9:45-10:15 | IL Ugrina |
| | | 10:15-10:30 | OP Kalebić | 10:15-10:30 | OP Labtim d.o.o. |
| 10:45-11:15 | *Coffee break* | 10:30-11:00 | *Coffee break* | 10:30-10:45 | CP Jasika d.o.o. |
| | *chair Novak Tušar* | | *chair Bosnar* | 10:45-11:00 | CP Alumina d.o.o. |
| 11:15-11:20 | CP Labtim d.o.o. | 11:00-11:15 | OP Hrenović | 11:00-11:15 | Closing remarks and *Coffee break* |
| 11:20-11:35 | OP Bosnar | 11:15-11:30 | OP Ivanković | | |
| 11:35-11:50 | OP Škrjanc | 11:30-11:45 | OP Dikić | | |
| 11:50-12:05 | OP Medak | 11:45-12:00 | OP Vukojević Medvidović | | |
| 12:05-12:20 | OP Palčić | 12:00-12:15 | OP Vukojević Medvidović | | |
| 12:20-12:35 | OP Rac | 12:15-12:30 | OP Mužek | | |
| | | 12:30-12:45 | OP Nuić | | |
| 12:35-14:00 | *Lunch* | 12:45-14:00 | *Lunch* | | |
| | *chair Zabukovec Logar* | | | | |
| 14:00-14:45 | PL Valtchev | | | | |

| | | | | |
|---|---|---|---|---|
| 14:45-15:15 | IL Markiv | | | PL = plenary lecture<br>IL = invited lecture<br>OP = oral presentation<br>CP = company presentation |
| 15:15-15:30 | OP Vu | | | |
| 15:30-15:45 |  *Coffee break* | | | |
| | *chair Trgo* | | | |
| 15:45-16:00 | OP Stojanović | | | |
| 16:00-16:15 | OP Pavlović | | | |
| 16:15-16:30 | OP Smiljanić | | | |
| 16:30-16:45 | OP Dimitrijević | 16:00-18:00 | *Diocletian Palace walking tour* | |
| 16:45-17:00 | OP Novaković | 19:00- | *Conference dinner* | |
| 17:00-17:15 | OP Dib | | | |

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# Boris Subotić: 75th anniversary of life and 52 years of scientific work

Dr. sc. Boris Subotić is a retired senior scientist at the Ruđer Bošković Institute. He is a well-known and internationally recognized scientist in the field of microporous and mesoporous materials, especially zeolites.

Dr. sc. Boris Subotić was born on December 02, 1946. in Dugo Selo, near Zagreb, Croatia. He achieved the BS degree at the Faculty of Science, University of Zagreb, and was employed in the Laboratory for Colloid Chemisty of the Ruđer Bošković Institute in 1969. In 1976 he obtained the Ph.D degree in Chemistry at the Ruđer Bošković Institute and the University of Zagreb. In 1988 he became a co-founder and member of the Laboratory for the Synthesis of New Materials, and from 1995 to 2011 he was the head of the same laboratory. He was also a member of the Institute's Scientific Council for a long time, and from 2005 to 2009 he was a member of director's advisory team and President of the Institute's Commission for Innovation. He was also co-founder of the Croatian Zeolite Association and the first president of the Association.

During his long scientific work, he established the research of zeolites at the Ruđer Bošković Institute (synthesis, characterization and application) with a special emphasis to investigation of mechanisms of crystallization and transformation of zeolites. In this context, he established theoretical basis of the model of autocatalytic nucleation of zeolites, and together with co-workers, experimentally proved validity and significance of the model. In the meantime, together with the coworkers, he developed the model of crystallization of zeolites based on the population balance theory, including all relevant sub-processes (mechanism and kinetics of precipitation amorphous aluminosilicate precursor, its dissolution as well as nucleation and crystal growth of zeolites). The results of more recent investigations showed that crystallization of zeolites in heterogeneous systems (aluminosilicate hydrogels) occur via formation/transformation of three different alumino-silicate precursors (gel, worm-like particles and condensed aggregates), and that crucial importance in the process of crystallization play the core-shell nano-precursors (3–20 nm in size) formed at the very early stage of the crystallization process. For this reason his very recent and present investigations are focused on the investigation of chemical and structural properties of the core (amorphous silica)@shell (TAA-polysilicates) and their influence on the course of crystallization and properties of products (zeolites).

*Dr. sc. Boris Subotić*

Besides the main directions of investigation, dr. Boris Subotić and co-workers also studied solution-mediated transformations of thermodynamically less stable types of zeolites (mainly zeolite A) and some other materials (orthombic barium fluoride) to more stable ones (zeolite P, hydroxysodalite, cubic zeolite P), thermodynamics and kinetics of exchange of cations from solution with the host ions from zeolites, mechanochemical transf-ormations of zeolites to amorphous phase, high-temperature transformations of zeolites, amorphous aluminosilicates to ceramics, etc.

Dr. sc. Boris Subotić is still very active scientist publishing regularly in prestigious world's journals.

He has published more than two hundred scientific papers, and between them more than hundred are included in Web of Science Core Collection. The number of citations of dr. sc. Boris Subotić is about 3000, and his h-index is 29. He is the author of five chapters in books and four patents. He has been the supervisor of one post-doctoral fellowship, ten PhD thesis and four master's theses.

Also for a great contribution in establishing of cooperation with many scientists, what is, in addition to scientific results, nice way to enrich knowledge of our and world's culture and art.

We wish him many years of good health and fruitful work.

Congratulations are joined by the Slovenian Zeolite Association and the Serbian Zeolite Association.

Tatjana Antonić Jelić (*Croatian Zeolite Association*)

# PREPARATION AND ANTIBACTERIAL ACTIVITY OF COMPOSITES BASED ON THYMOL/CARVACROL AND CLINOPTILOLITE

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## ABSTRACT

Composites based on clinoptilolite and monoterpene phenols - thymol and carvacrol were prepared by supercritical solvent impregnation (SSI) at 30 MPa and 35 ºC during 18 h in supercritical carbon dioxide ($scCO_2$). The composites were characterized in detail and their antibacterial activity was tested towards two potentially pathogenic bacteria; *Escherichia coli* and *Staphylococcus aureus*.

Key words: thymol, carvacrol, antibacterial activity, clinoptilolite, supercritical solvent impregnation.

## INTRODUCTION

Due to antimicrobial properties, phenols and polyphenols obtained from plant essential oils are intensively studied. Thymol [2-Isopropyl-5-methylphenol] and carvacrol [5-Isopropyl-2-methylphenol] are major components of oregano (*Origanum vulgare*) used from ancient times [1]. Both compounds exhibit antimicrobial, antioxidant, anti-inflammatory, antitumor, antimutagenic, analgesic, anti-parasitic and insecticidal properties [1,2]. The use of bioactive compounds is most favourable if they are immobilized on suitable carriers. In this work natural zeolite – clinoptilolite was tested as a carrier of thymol and carvacrol, and antibacterial activity of the prepared composites towards Gram negative *Escherichia coli* DSM 498 and Gram positive *Staphylococcus aureus* ATCC 25923 were studied.

## EXPERIMENTAL

*Preparation of the composites*

Zeolitic tuff (Z) with about 70 wt. % of natural zeolite – clinoptilolite from Vranjska Banja deposit (Serbia) was used in experiments. Z was converted into $NH_4$-form ($NH_4$-Z) using a solution of ammonia acetate (1 mol $dm^{-3}$) and H-form (H-Z) using the following procedure. $NH_4$-Z was firstly calcined in air at 550 ºC and the calcined product was treated with 0.6 mol $dm^{-3}$ HCl at 70 ºC. The obtained product was washed with distilled water until the negative reaction to $Cl^-$ and dried overnight at 60 ºC to a constant mass.

Crystalline thymol and liquid carvacrol (purity > 99%, Sigma Aldrich, Germany) were impregnated onto H-Z in a high-pressure view cell using a static mode at optimized conditions (35 ºC, 30 MPa, time interval of 18 h, decompression rate of 1.5 MPa $min^{-1}$) using $CO_2$ as supercritical fluid. The obtained composites were denoted as T-Z (thymol-containing zeolite) and C-Z (carvacrol-containing zeolite).

*Characterization of synthetized composites*

The content of thymol and carvacrol in composites were determined by thermogravimetric analysis (TGA) using a SDT Q-600 simultaneous DSC-TGA instrument (TA Instruments), as well as by C, H, N analysis using Varian EL III C,H,N,S/O Elemental Analyzer (Elementar, Langenselbold, Hesse, Germany). Crystallinity of the samples was examined by PXRD method using Ultima IV Rigaku diffractometer equipped with Cu $K_{\alpha 1,2}$

radiation using a generator voltage (40.0 kV) and a generator current (40.0 mA). The PXRD patterns were recorded in the 2θ range 5 – 45° with a scanning step of 0.02 ° and the scan rate of 5 °min$^{-1}$. Specific surface area was measured by $N_2$ adsorption–desorption experiments (Micromeritics ASAP 2020) and calculated according to the Brunauer, Emmett, Teller (BET) method. Interactions of the phenols and zeolite lattice were studied by Fourier Transform Infrared (FTIR) Spectroscopy. The FTIR spectra were recorded in the range 4000–450 cm$^{-1}$ with a resolution of 4 cm$^{-1}$ at room temperature, using Nicolet iS10 (Thermo Scientific) spectrometer.

*Antibacterial activity test*

Antibacterial activity of the composites was tested in different water media: phosphate buffer solution (PBS), commercially available spring water (Gala, Serbia, SW), and lake water (Sava Lake, Belgrade, Serbia, SL) toward Gram negative *E. coli* DSM 498 and Gram positive *S. aureus* ATCC 25923. Firstly, bacteria were pre-grown on the Nutrient agar (NA, Torlak, Serbia) for 16 h at 37±0.1 ºC to obtain cultures in a *log* phase of growth. All water media were sterilized before the tests by autoclaving (121 ºC/20 min) and the composite samples by UV light in UV chamber for 30 min.

The experiments were performed as follows: into 10 cm$^3$ of bacterial biomass suspended in a sterile water media 0.1 g of each composite (T-Z or C-Z) was added, and incubated in a thermostatic water bath during 24 h at 37±0.1º C with shaking at 105 rpm. As a positive control (without antibacterial activity), the bottles with 0.1 g of H-Z in all studied media with bacteria were set up. The number of viable bacterial cells was determined at the beginning of the experiment, after 1 h (short contact) and after 24 h (long contact). After 1 and 24 h the aliquot of 0.1 cm$^3$ was plated by a spread plate method directly on NA and another amount of the sample (1 cm$^3$) was serially diluted ($10^{-1}$–$10^{-7}$). Diluted samples have also been plated onto NA and incubated for 24 h at 37±0.1º C. After incubation the bacterial colonies grown on NA were counted. The number of bacteria was reported as CFU (Colony Forming Units) per one cm$^3$, logarithmically transformed and the antibacterial activity was finally expressed as the percent of reduction of the log of CFU cm$^{-3}$ according to the Equation (1). All experiments were done in triplicate.

$$Reduction\ (\%) = \frac{\log CFU\ cm^{-3}\ (t_0) - \log CFU\ cm^{-3}\ (t)}{\log CFU\ cm^{-3}\ (t_0)}\ x\ 100 \qquad (1)$$

where $t_0$ presents the initial number of bacteria and $t$ is the number of bacteria after time of contact (1 or 24 h).

*Desorption of thymol and carvacrol from composites*

Leaching of thymol and carvacrol from the composites were studied after antibacterial activity tests. The concentration of the phenols was measured photometrically using a UV-VIS spectrophotometer Cary 100 Scan (Varian) at $\lambda_{max}$= 274 nm.

## RESULTS AND DISCUSSION

The conversion of Z into H-Z significantly increased the specific surface area of Z from 42 m$^2$ g$^{-1}$ to 230 m$^2$ g$^{-1}$ (H-Z) suggesting that the modification led to a partial pore opening of clinoptilolite lattice. PXRD analysis (not shown) confirmed that the modification is not accompanied by loss of zeolite crystallinity.

Thermal properties of the phenols and composites are presented in Fig.1. Thermogram of H-Z shows rather continual weight loss (10.0 wt.%) up to 350 °C. The thermal decomposition of thymol and carvacrol (not shown in the Figure) proceeds as one-step-process up to 160 °C with DTG maxima centred at 119 (thymol) and 148 °C (carvacrol). Fig. 1 shows that the composites have similar thermal behaviour: T-Z displays a strong DTG maximum at 134 and

C-Z at 135 °C, indicating that the SSI procedure successfully loaded phenols onto H-Z. Thymol and carvacrol content obtained from TGA agrees well with C, H, N analysis: 23.0 wt.% of thymol was obtained for T-Z and 19.2 wt.% of carvacrol for C-Z.

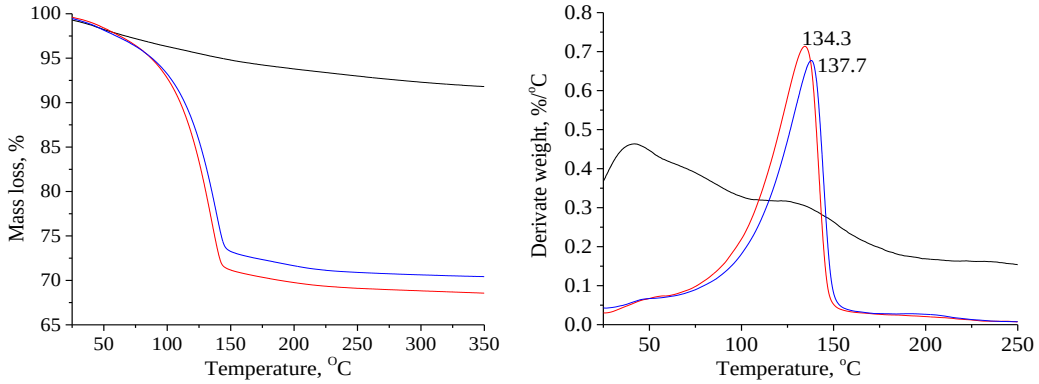


Figure 1. TG and DTG curves of H-Z (black line), T-Z (red line) and C-Z (blue line)

Fig. 2 shows FTIR spectra, which confirm the presence of the phenols on the composites. A broad band at 3580 – 3160 $cm^{-1}$ corresponds to the phenolic –OH stretching vibrations, bands at 3000 – 2850 $cm^{-1}$ belong to the C-H symmetric and asymmetric stretching vibration [3], while the bands between 1620 and 1417 $cm^{-1}$ originate from C=C stretching vibrations in phenolic ring of thymol and carvacrol [4]. The bands attributed to the out-of-plane C-H wagging and bending vibrations from isoprenoids at around 804 $cm^{-1}$ and at 945 $cm^{-1}$, respectively, were ascribed to phenols presented in the zeolite lattice [5]. Taking into account band positions it can be concluded that there is no significant difference between free and immobilized phenols suggesting that the interactions of the phenols and Z do not include formation of covalent bonds.

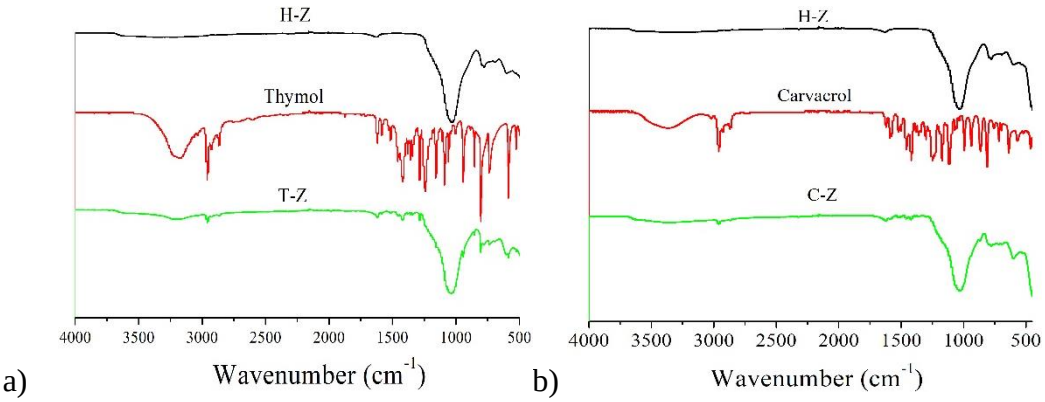


Figure 2. FTIR spectra of H-Z, thymol and T-Z (a) and H-Z, carvacrol and C-Z (b).

*Antibacterial activity*

Antibacterial activity was investigated toward Gram negative *E. coli* and Gram positive *S. aureus* in different water media (Table 1.). According to previous studies, H-Z did not exhibit any antibacterial activity towards examined strains (data not shown). After 1 h of contact in SL, T-Z exhibited a significant antibacterial activity toward both strains (62.6 and 72.2 % toward *E. coli* and *S. aureus*, respectively). In other water media, T-Z exhibited bactericidal effect after only 1 h of contact, except in the case of *S. aureus* in SW, where bactericidal effect appeared after 24 h. The C-Z exhibited bactericidal activity toward both examined strains in all media after 1 h of contact. Considering the obtained results, it is evident that C-Z shows better antibacterial activity than T-Z in the case of short-term exposure. This suggests that mechanism of the antibacterial action is influenced by chemical structure of bioactive compound.

Table 1. Percent of reduction in number of *E. coli* and *S. aureus* after 1 and 24h of contact with T-Z and C-Z in different water media.

| Water medium | Reduction (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | T-Z | | | | C-Z | | | |
| | *E. coli* | | *S. aureus* | | *E. coli* | | *S. aureus* | |
| | 1 h | 24 h | 1 h | 24 h | 1 h | 24 h | 1 h | 24 h |
| PBS | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 |
| SW | 100±0 | 100±0 | 70±0 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 |
| SL | 65±7 | 100±0 | 71±2 | 100±0 | 100±0 | 100±0 | 100±0 | 100±0 |

$t_o$ *E.coli* 2.1 x $10^7$ CFU $cm^{-3}$; *S. aureus* 7.0 x $10^6$ CFU $cm^{-3}$ (experiment with thymol)
$t_o$ *E.coli* 7.6 x $10^6$ CFU $cm^{-3}$; *S. aureus* 1.5 x $10^7$ CFU $cm^{-3}$(experiment with carvacrol)

*Desorption of thymol and carvacrol from the composites*

Leaching of thymol and carvacrol from the T-Z and C-Z were determined in all water media after antibacterial tests (Table 2). Although antibacterial activity of C-Z is more pronounced than that of T-Z, the amount of thymol leached from T-Z is significantly higher than carvacrol leached from C-Z. This could be explained by weaker interactions between thymol and zeolite.

Table 2. Percentage of the leached thymol/carvacrol from the composites T-Z and C-Z after 24 h of the contact.

| Water media | % of the leached phenols | | | |
|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *E. coli* | *S. aureus* |
| | Thymol | Carvacrol | Thymol | Carvacrol |
| PBS | 23.2 | 8.6 | 22.7 | 6.2 |
| SW | 26.3 | 7.2 | 26.1 | 8.1 |
| SL | 32.9 | 8.6 | 28.1 | 8.9 |

## CONCLUSION

Components of the essential oils such as thymol and carvacrol can be successfully immobilized onto natural zeolite - clinoptilolite by the supercritical solvent impregnation. The composites that contained 23.0 wt.% of thymol and 19.2 wt.% of carvacrol showed bactericidal activity toward both Gram-negative *E. coli* and Gram-positive *S. aureus*. This suggests their potential use as disinfectant agents. It is worth noticing that the impregnation of these phenols by supercritical solvent impregnation used in this work is a novel and an environmentally friendly approach in the preparation of materials with antibacterial properties.

## ACKNOWLEDGEMENTS

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